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Imbalanced serum IgG subclass pattern in toxic shock syndrome patients: deficiency of specific IgG1 and IgG4 subclass antibodies to toxic shock syndrome toxin 1

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SUMMARY

An imbalanced serum IgG subclass pattern was identified in 10 patients with toxic shock syndrome (TSS) showing remarkably low subclass levels of various combinations. IgG2 levels were significantly reduced as compared to normal controls. The IgG subclass-specificity of antibodies to toxic shock syndrome toxin (TSST-1) was investigated by a solid-phase radioimmunoassay. TSS-patients lacked pre-immunity to TSST-1 in all four IgG subclasses. Normally acquired immunity to the toxin as well as the serological response developing in two patients with TSS was generally restricted to IgG1 and IgG4. A strong booster response of all four IgG subclasses was seen in three patients with *S. aureus* septicaemia due to TSST-1 producing strains. The lack of specific IgG1 and IgG4 antibodies to TSST-1 and the low serum IgG subclass levels found in the TSS-patients could be of pathogenetic significance and help to explain the susceptibility to TSS in certain individuals.

Keywords IgG subclass toxic shock syndrome TSST-1

INTRODUCTION

Toxic shock syndrome (TSS) was first described by Todd *et al.* (1978); later, Bergdoll *et al.* (1981) and Schlievert *et al.* (1981) independently showed a *Staphylococcus aureus* exoprotein to be associated with the syndrome. This protein was assigned as either staphylococcal enterotoxin F (Bergdoll *et al.* 1981) or pyrogenic exotoxin C (Schlievert *et al.*, 1981) but they were later shown to be identical (Bonventre *et al.*, 1983) or at least very similar (Cohen *et al.*, 1983) and are now referred to as Toxic Shock Syndrome Toxin 1 (TSST-1) (Bergdoll & Schlievert, 1984).

Healthy adults have a seropositive reaction to the toxin in 78–95% as determined by radio-immunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) (Bergdoll *et al.*, 1981; 1982; Notermans *et al.*, 1983; Vergeront *et al.*, 1983; Christensson & Hedström, 1985). Patients acquiring TSS initially generally lack or show a very low level of antibodies to TSST-1 (Bergdoll *et al.*, 1981; 1982; Notermans *et al.*, 1983; Vergeront *et al.*, 1983; Bonventre *et al.*, 1984; Chesney *et al.*, 1984; Christensson & Hedström, 1985; Stolz *et al.*, 1985). Furthermore, patients with menstrual-related TSS are often subject to recurrencies of the infection still without evidence of specific antibody response against TSST-1 (Chesney *et al.*, 1984; Stolz *et al.*, 1985). Various suggestions for this lack

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of immune response have been presented (Stolz *et al.*, 1985), but there is as yet no evidence for a specific immunodeficiency in TSS-patients. In the present studies we have determined serum IgG subclass levels in patients with TSS and investigated the IgG subclass specificity of anti-TSST-1 antibodies by solid phase radioimmunoassay (SPRIA).

MATERIALS AND METHODS

Patients and controls. All sera were collected at the Department of Infectious Diseases, Lund, Sweden, except for sera for determination of normal subclass levels, which were obtained from the Department of Pediatrics at Lund. All individuals were above 15 years of age as shown in Table 1.

Serum samples from the TSS patients were collected during the acute illness, one to three days after onset of infection, and during convalescence after 25–60 days. Both serum IgG subclass levels and IgG subclass specific antibodies to TSST-1 were determined. All TSS patients fulfilled the criteria of TSS (Centers for Disease Control, 1980; 1982). Five patients had menstrual-related TSS and two of these patients were investigated during recurrencies of the infection. Four of the five non-menstrual related TSS patients were males and all five had cutaneous *S. aureus* infections.

Diagnostic criteria of *S. aureus* endocarditis and septicaemia were as previously described (Christensson *et al.*, 1985). Acute serum samples from the first week of infection together with convalescent sera ranging from 14 days to seven months after onset of infection were investigated in the IgG subclass specific anti-TSST-1 SPRIA. Four of the 16 *S. aureus* septicaemia patients were infected with TSST-1 producing strains. TSST-1 production was kindly determined by P. Schlievert, Minneapolis, USA (Schlievert *et al.*, 1981).

Single sera from two groups of healthy controls were examined in the assay of serum IgG subclass levels (group I) and the subclass specific SPRIA (group II), respectively (Table 1).

Assay of human IgG subclasses. Serum IgG subclass levels were examined by electroimmunoassay according to Oxelius (1978).

Monoclonal antibodies. Commercially available mouse monoclonal antibodies against the four human IgG subclasses and total IgG were purchased from Seward Laboratory, London, England. The monoclonals against IgG1 (BAM 15), IgG2 (BAM 10), IgG3 (BAM 8), IgG4 (BAM 11) and total IgG (BAM 6) were purified from ascitic fluid by High Performance Liquid Chromatography. The protein concentrations were determined spectrophotometrically at 280 and 310 nm. Ten μg from each of the five purified myeloma proteins were radiolabelled with ^{125}I (The Radiochemical Centre, Amersham, England) to a specific activity of 5 μCi/μg using a lactoperoxidase method (Thorell & Johansson, 1971).

IgG subclass-specific TSST-1 SPRIA. The wells of PVC Titertek Immunoassay plates (Flow Lab., Netherlands) were coated with 100 μl of TSST-1 at a concentration of 0·5 μg/ml diluted in 0·1

Table 1. Number, age and sex of patients and controls

Diagnosis	Age		Sex
	Range	Mean	M/F
TSS ($n=10$)	16–66	33	4/6
S. aureus septicaemia or endocarditis ($n=16$)	16–81	56	9/7
Healthy controls I ($n=27$)	15–72	39	11/16
Healthy controls II ($n=10$)	15–45	32	5/5

M/F, male/female.

M glycine buffer, pH 9·5. TSST-1 was kindly provided by M. Bergdoll, Wisconsin, USA (Reiser *et al.*, 1983). After incubation at 4°C overnight plates were washed in PBS-T (PBS with 0·05% Tween 20). A blocking buffer (100 μl) containing PBS-merthiolate with 4% bovine serum albumin (Sigma Chemical Co., St Louis, MO) and 0·2% gelatin was added for 2 h at room temperature and overnight at 4°C. After washing with PBS-T 100 μl serum diluted 1/100 in the blocking buffer was incubated for 2 h at room temperature on a microtitre plate shaker (Bellco Glass, Inc., Vineland, NJ). After washing, 5 ng ^{125}I-labelled monoclonal antibodies against IgG subclass 1, 2, 3, 4 and total IgG, diluted in 100 μl PBS with 0·2% gelatin, was added for another 2 h at room temperature on the microtitre plate shaker. After a final washing procedure the wells were cut apart, placed in plastic tubes and counted in a gamma counter (LKB Wallac 1870, Rackgamma). The optimal antigen and serum dilutions were established by checkerboard titrations.

All sera were tested in duplicate and a positive and negative control serum were investigated on each plate. Results are given as net ct/min values, where ct/min values obtained with sera incubated in wells lacking the antigen (corresponding to non-specific adsorption to the plastic) have been subtracted.

The reactivity of the monoclonal antibody assays was assured by the reaction with polyclonal IgG (Gammaglobulin, KABI, Stockholm, Sweden).

Statistical analysis. Wilcoxon's two-tailed rank sum test was used for comparison of antibody levels between patient and control groups.

RESULTS

Serum IgG subclass levels. The 10 TSS-patients had significantly lower levels of IgG2 as compared to the controls ($P < 0·001$), (Fig. 1). Two patients had low levels of one IgG subclass (G2; G3), four patients of two subclasses (G1,2; G1,3; G2,3; G2,4), and four patients of three subclasses (G1,2,3). Since the normal range of IgG4 levels is very wide (including the non-measurable levels below 0·01 g/l), the results of the IgG4 levels in the TSS-patients are difficult to interpret. There was no difference between the total serum IgG levels in acute serum samples (mean value 9·36 g/l; range 6·12–16·40 g/l) and convalescent sera (mean value 10·19 g/l; range 6·07–16·46 g/l) from the TSS patients ($P > 0·05$). However, total serum IgG levels in acute sera from the TSS patients were lower than those of the healthy controls (mean value 12·07 g/l; range 7·85–18·43 g/l), ($P < 0·05$).

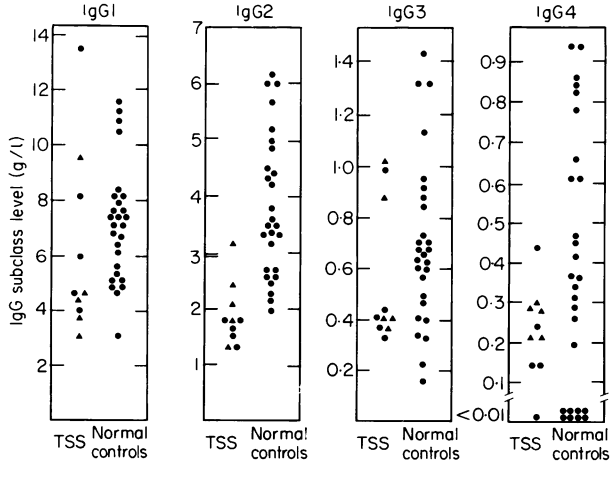

Fig. 1. IgG subclass levels in acute sera from 10 TSS-patients and 27 normal controls. Non-menstrual associated TSS, (▲); menstrual associated TSS, (●).

IgG subclass specific TSST-1 antibodies. There was a good correlation between the total specific IgG antibody response to TSST-1 measured in the SPRIA and that of a previously presented ELISA (Christensson & Hedström, 1985) (Spearman rank correlation coefficient $r_s = 0{\cdot}92$, $P < 0{\cdot}001$) (data not shown). The reproducibility of the SPRIA was satisfactory, with an analytical error of the method of ± 50 ct/min within the range 0–200 ct/min, and values below 50 ct/min were considered negative (Fig. 2 A–C). All subclass-specific monoclonal antibodies showed a reactivity against commercial gammaglobulin.

All 10 TSS patients showed negative subclass specific antibodies in their acute sera. Only two non-menstrual related TSS patients had positive convalescent titres and the antibody response to TSST-1 was restricted to IgG1 and IgG4 (Fig. 2A).

No titre rises were seen in the *S. aureus* endocarditis or septicaemia patients not infected with TSST-1 producing strains, and the convalescent antibody levels are shown in Fig. 2B. TSST-1 antibodies were mainly found in subclasses IgG1 and IgG4, and the same subclass specificity was seen among the healthy controls (Fig. 2C). One septicaemia patient and one healthy control individual also showed a positive but low antibody level in IgG2 (Fig. 2B and 2C).

The subclass specificity in *S. aureus* septicaemia patients infected with TSST-1 producing strains showed a different pattern. Three of these four patients had positive antibody levels in IgG1 and

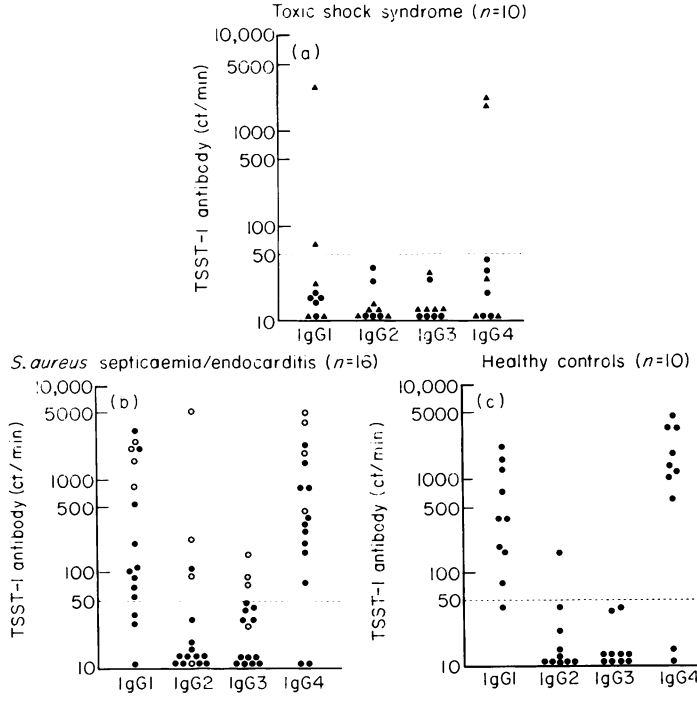

Fig. 2. (a) Subclass-specific IgG antibodies to TSST-1 in patients with TSS as determined by SPRIA. Convalescent titres 25–60 days after onset of infection are expressed as counts per minute values (ct/min). Non-menstrual associated TSS, (▲); menstrual associated TSS, (●). (b) Subclass-specific IgG antibodies to TSST-1 in patients with *S. aureus* septicaemia or endocarditis as determined by SPRIA. Convalescent antibody levels 14 days to 7 months after onset of infection are expressed as counts per minute values (ct/min). Septicaemia due to TSST-1 producing strain (✪). (c) Subclass-specific IgG antibodies to TSST-1 in healthy controls as determined by SPRIA and expressed as counts per minute values (ct/min).

IgG4 at the onset of septicaemia and they developed a booster response in all four IgG subclasses (Fig. 2B). The fourth patient, a 16-year-old girl with no pre-existing antibody level, developed a serological response in IgG1 and IgG4. None of these four septicaemia patients showed any signs of TSS.

DISCUSSION

There is indirect evidence that antibodies to TSST-1 protect against the development of TSS. Firstly, most TSS patients lack pre-existing specific antibodies (Bergdoll *et al.*, 1981; 1982; Notermans *et al.*, 1983; Vergeront *et al.*, 1983; Bonventre *et al.*, 1984; Chesney *et al.*, 1984; Christensson & Hedström, 1985; Stolz *et al.*, 1985), and secondly, these antibodies measured by ELISA correlate well with antibodies neutralizing the TSST-1 induced release of interleukin-1 (Hirose-Kumagai *et al.*, 1984). Moreover, antibodies to TSST-1 were recently shown to be protective in a rabbit model (Melish *et al.*, 1985).

Many theories have been put forward as to why certain TSS-patients fail to produce specific antibodies to TSST-1 when the majority of healthy adults show serological immunity to the toxin. The alteration of lymphocyte function induced by the toxin (Schlievert, 1983; Bergdoll & Schlievert, 1984) and the lymphopenia seen in acutely ill TSS-patients (Bergdoll & Schlievert, 1984; Stolz *et al.*, 1985) have been suggested to play a role. The size as well as the amount of the toxin has been proposed to be too small to be recognized by the immune system (Schlievert, 1983; Stolz *et al.*, 1985). A specific unresponsiveness rather than a generalized immunodeficiency has also been suggested in a rabbit model (Schlievert, 1983). Furthermore, TSS-patients in general do not present a previous history of unusual susceptibility to other severe infections (Davis, Vergeront & Chesney, 1982), and therefore a generalized immunodeficiency has not been a likely explanation.

In the present investigations we did not find a total deficiency of any IgG subclass among the TSS-patients, but remarkably low levels of several IgG subclasses, where IgG2 was significantly reduced in the TSS-group. Furthermore, total serum IgG was lower in the TSS group, and showed no rise during convalescence. The low levels of serum IgG subclasses could not be explained by differences in age and sex between TSS patients and controls (Table 1), since the individuals in both groups were well above the age where adult IgG subclass levels should be expected (Oxelius, 1979). The normally acquired immunity to TSST-1 as well as the specific IgG response eventually developing in two TSS patients was generally not found in IgG2 but rather in IgG1 and IgG4. This is in accordance with previous investigations, where the immune response against protein antigens generally was restricted to IgG1, IgG3 and IgG4 (for review see Shakib & Stanworth, 1980; Hammarström *et al.*, 1984). It has, however, previously been shown, that some IgG2 deficient sera also lacked specific antibodies to protein antigens in the IgG1 and IgG4 subclasses (Hammarström *et al.*, 1984). The imbalanced serum IgG subclass pattern, mainly with low IgG2 levels, found in our TSS patients could therefore explain the lack of specific anti-TSST-1 antibodies in IgG1 and IgG4. Because these specific antibodies are probably protective against TSS, the IgG subclass imbalance could be of pathogenetic significance in explaining the development of TSS in certain individuals.

The lack of TSS symptoms in the three pre-immune patients with septicaemia due to TSST-1 producing strains further indicate, that specific IgG1 and IgG4 antibodies to TSST-1 are protective. One may speculate, that their strong booster response of TSST-1 antibody developing in all four IgG subclasses may have been due to the severity of the infection.

It is noteworthy, that the young female, who lacked preimmunity to TSST-1 when acquiring septicaemia due to a toxin-producing strain, did not show any symptoms of TSS. Instead, she eventually developed a specific IgG1 and IgG4 antibody response to TSST-1. This could imply that other factors than humoral immunity to TSST-1 are important in whether TSS will develop in a patient challenged with a toxin-producing strain. The localization of the infection probably plays a role, as menstrually associated infections are the most common (Chesney *et al.*, 1984). Though as many as 18% of 88 *S. aureus* septicaemia strains were TSST-1 producers in a 5-year *S. aureus* septicaemia material from the University Hospital of Lund, Sweden (Christensson & Hedström, 1986), septicaemia-associated TSS seemed to be rare. Only one of 169 *S. aureus* septicaemia patients

had symptoms of TSS during the 5-year period between 1976 and 1980 (unpublished results). Unfortunately no serum samples were available from this patient.

In conclusion, we found an imbalanced serum IgG subclass pattern in TSS-patients together with a lack of specific, probably protective, IgG1 and IgG4 antibodies against TSST-1. These observations could help to explain the susceptibility to TSS in certain individuals.

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